INFLUENCE AND INTERACTION OF DIFFERENT PARAMETERS ON THE SURVIVAL OF TWO STRAINS OF *SALMONELLA ENTERICA* IN PIG SLURRY

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Introduction

Excretion of *Salmonella enterica* by late-fattening pigs may cause the dissemination of this zoonotic agent in the environment during spreading of contaminated slurry (Suraj B. Baloda et al., 2001). Slurry spreading on pastures represents a risk of contamination of animals (ruminants), and also pollution (Baudart J. et al., 2000). Even though studies have shown the purifying role of slurry (Placha I. et al. 2001), persistence of *Salmonella* in the soil after slurry spreading has been demonstrated (Suraj B. Baloda et al., 2001). Controlling the risk related to the spreading of this effluent entails describing the influence and interaction of parameters on the survival of *Salmonella* in pig slurry. A preliminary study is carried out to compare the mini-MSRV MPN technique (Fravalo P. et al., 2003) to direct isolations on brilliant green agar supplemented with rifampicin. Then experimental designs are developed using Doelhert uniform shell design (Doelhert D.H. 1971) in order to study the effect of different parameters on the survival of *Salmonella enterica* in pig slurry. The objective of this study is to describe the influence and interaction of different factors on the survival of two strains of *Salmonella enterica* in pig slurry.

Material and methods

Pig slurry controlled free of *Salmonella enterica* is placed into four flasks amended with four rifampicin-resistant *Salmonella* strains (*Salmonella* t*yphimurium*, *Salmonella brandenburg*, *Salmonella* d*erby*, *Salmonella infantis*). *Salmonella* counting is done every 3 or 4 days during 28 days. Using rifampicin-resistant strains makes it possible to compare the mini-MSRV MPN technique to direct isolations on brilliant green agar supplemented with rifampicin. Two experimental designs are developed using Doelhert uniform shell design. Three parameters are studied at different levels: initial *Salmonella* concentration (3 levels), slurry storage temperature (5 levels), and slurry storage time (7 levels from 0 to 6 days). Two *Salmonella* strains are used: *Salmonella* t*yphimurium* and multiresistant *Salmonella*

t*yphimurium* DT 104 (resistance to ampicillin, chloramphenicol, streptomycin, sulphonamides, and tetracyclines). The number of experiences to be performed is determined according to the following formula: $N=k^2+k+1+n$ (k = number of parameters studied; n = number of replications at the centre of the model); i.e., 16 experiments per strain and per experimental design. This mathematical tool is chosen to replace the traditional method in order to study interactions between factors and to obtain maximum information and precision from a reduced number of experiments. Flasks are filled with 200 mL slurry controlled free of *Salmonella*, amended with *Salmonella* and stored under the conditions set by the experimental designs. Results are expressed as *Salmonella* counting, done by mini-MSRV MPN technique, at the appropriate time set by the experimental designs.

Results

In the preliminary study, comparing count means in paired series ($p<0.05$) bring evidence that mini-MSRV MPN technique is adapted to count *Salmonella* in pig slurry. Moreover, mini-MSRV MPN technique counting is possible with concentrations lower than the limit of numeration by direct isolation.

After processing results of the experimental designs using STATGRAPHICS® software, we get the significance (P value) that is set at the limit of 5% and the effects assessed for each parameter, as well as the effect of their interactions on the decrease in the amount of slurry *Salmonella* (table 1).

Table1. Results of experimental designs; ST: S. typhimurium; ST104: S. typhimurium DT104.

	Parameters	P value ST	Effects	P value ST 104	Effects
	Average		3.645		3.660
Parameters	A: Temperature	0.020	- 1.160	0.004	- 1.370
	B: Time	0.035	- 0.818	0.053	- 0.455
	C: Concentration	0.005	1.530	0.001	1.667
Interaction between parameters	AA	0.082	- 1.150	0.016	- 1.440
	BB	0.064	- 0.958	0.186	- 0.375
	CC	0.010	- 1.662	0.010	- 1.063
	AB	0.013	- 2.750	0.020	- 1.530
	AC	0.508	0.407	0.070	0.980
	BC	0.690	- 0.207	0.445	0.270

For the two strains, storage temperature clearly showed a significant negative effect and initial concentration has a positive effect. Time storage is significant only for *Salmonella*

typhimurium with a negative effect. Temperature/time interaction has a significant negative effect for both strains.

Discussion and conclusion

The *Salmonella* initial concentration is essential in a forecast objective. Mini-MSRV MPN technique, validated in this study, appears as an interesting counting tool for *Salmonella* in pig slurry. It is reliable, easy to operate and inexpensive.

Temperature storage and initial concentration are parameters that influence the survival of the two strains. Time storage of *Salmonella typhimurium* DT104 has a P value (0.053) close to the limit of significance (0.05). Furthermore the effect of temperature/time is significant for the both strains.

The three parameters showed a real effect on the survival of these strains of Salmonella *enterica* in pig slurry. These results are similar to those reported by different authors in their experiments or in natural contaminations (Placha et al 2001,Watabe et al 2003). The time/temperature interaction should be included in further studies to obtain a model of the *Salmonella* decrease as function of data collected in herd.

Given our results and those describe by other authors, the slurry storage time will depend on the season (longest in winter), the initial *Salmonella* concentration and the strain.

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References

1. *Baudart J., Lemarchand K, Brisabois A., Labaron P. Diversity of Salmonella Strains isolated from aquatic environment as determined by serotyping and amplification of the ribosomal DNA spacer regions. Applied and Envir Microbiol 2000, 1544-1552.*
2. *Doelhert D.H. Applied statistics 1970,19,231-239.*
3. *Fravalo P, Hascoët Y, Le Fellic M, Queguiner S, Petton J, Salvat G. Convenient method for rapid and quantitative assessment of Salmonella enterica contamination : the mini-MSRV MPN Technique. J Rapid Methods Automation Microbiol 2003,11,81-88.*
4. *Placha I, Venglovský J, Sasáková N, Svoboda IF. The effect of summer and winter seasons on the survival of Salmonella Typhimurium and indicator micro-organisms during the storage of solid fraction of pig slurry. J Appl. Microbiol 2001,91,1036-1043.*
5. *Suraj B. Baloda, Lise Christensen, Silvija Trajcevska. Persistence of a Salmonella enterica Serovar Typhimurium DT12 clone in a piggery and in Agricultural Soil amended with Salmonella-Contaminated Slurry. Applied and Env Microbiol, 2001,2859-2862.*
6. *Watabe M., Rao JR, Stewart TA, Xu J, Millar BC, Xiao L, Lowery CJ, Dooley JSG, Moore JE. Prevalence of bacterial faecal pathogens in separated und unseparated stored pig slurry. Applied Microbiol 2003, 36, 208-212.*